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OPPTS Ecologic Effects Test Guidelines Series 850

Introduction:

OPP and OPPT have agreed on a series of test guidelines which may be used to develop ecological effects data in support of FIFRA and TSCA requirements. The attached Master List for the 850 series identifies the OPP, OPPT, or OECD sources from which these guidelines were derived. Guidelines from both programs have been fully harmonized. In addition, OPPTS guidelines have been harmonized with their OECD counterparts, where applicable. Four guidelines 850.1075, 850.1400, 850.1730, and 850.6800 are fully harmonized with their OECD counterparts. An additional five OPPTS guidelines with OECD counterparts, 850.1010, 850.1300, 850.2200, 850.2300, and 850.5400, were not harmonized fully with OECD guidelines because key aspects of the current OECD guidelines were deemed inappropriate to meet OPPTS needs. The United States and other OECD member nations have identified these guidelines to the OECD for revision or updating. For example, in one case, the OPPTS guideline for the Daphnid Chronic test (850.1300) is harmonized with the new draft OECD guideline rather than the existing one.

Honeybee Testing:

The guidelines for honeybee toxicity testing are all unique to OPP. These were revised and clarified by addition of Data Reporting Guidance and material from the Standard Evaluation Procedures. In addition, a new test method was provided for the Honeybee Acute Toxicity test.

Nontarget Plant Testing:

A large proportion of the guidelines for nontarget plants are now fully harmonized within OPPTS. Where possible, plant guidelines were harmonized with OECD. However, many of the OECD guidelines were deemed unsuitable for use in North America. OECD has now identified the fact that its nontarget plant testing guidelines may need significant changes to accommodate the needs of testing pesticides as well as industrial chemicals. Work has been initiated in this area in which the United States is a participant.

The guidelines for nontarget plants have been expanded and updated considerably. A task force in OPP had identified a large number of issues for nontarget plant testing which needed clarification or resolution. The Rejection Rate project provided an opportunity for Agency

recommendations to be fully discussed with industry. The changes to the nontarget plant test guidelines address such areas as more precise definitions, upgraded references, including new ASTM methods (algal toxicity and *Lemna* testing), and improved or refined guidance on test methods and procedures.

Avian and Terrestrial Species:

Most guidelines for avian and terrestrial testing benefitted from the cross-fertilization of harmonization. In the case of avian reproduction testing, OPPTS has deferred full harmonization of all species until OECD improves its own test methodology for Japanese quail. (The current test guideline identifies Japanese quail, but does not provide scientifically feasible guidance for the test.) In general, the guidance for both bobwhite quail and mallard duck have been harmonized with OECD guidelines for avian reproduction testing.

850.1000 Special Considerations for Conducting Aquatic Laboratory Studies

General guidance primarily for dealing with difficult to test substances and analytical verification of test concentrations. The guidance provided is an outgrowth of recommendations from a joint industry and Agency workgroup to address aquatic testing issues.

850.1010 Aquatic Invertebrate Acute Toxicity, Freshwater Daphnids

This guideline is based on existing OPPTS (40 CFR 797.1300) and OPP (72-2) guidelines with no substantive differences. It differs from OECD guideline 202 principally in the prescribed test duration. OECD 202, Part I, describes a short-term range-finding test that is intended for use with a much longer reproduction test. This range-finding test currently allows a 24 hour test duration. That 24-hour duration is inconsistent with most other published testing methods for determining acute toxicity of chemicals to daphnids. OPPTS guideline 850.1010 is consistent with the existing OPPTS and OPP guidelines which require a 48 hour duration. OECD is expected to revise their guideline (202 Part I) to also require a 48 hour duration. Approval for this

work has been "given priority" by the National Coordinators, OECD Test Guidelines Programme, at the 6th National Coordinators Meeting. This planned revision was characterized as "editorial work" by the National Coordinators and should be included in the Dec 95-Sep 96 work plan of the OECD Test Guidelines Programme.

850.1020 Gammarid Acute Toxicity Test

This guideline is substantively identical to 40 CFR 795.120 and is unique to OPPTS.

850.1025 Oyster Acute Toxicity Test (Shell Deposition)

This guideline is based on 40 CFR 797.1800 and OPP 72-3 and is fully harmonized within OPPTS.

850.1035 Mysid Acute Toxicity Test

This guideline is based on 40 CFR 797.1930 and OPP 72-3 and is fully harmonized within OPPTS.

850.1045 Penaeid Acute Toxicity Test

This guideline is based on 40 CFR 797.1970 and OPP 72-3 and has been harmonized for OPPTS.

850.1055 Bivalve Acute Toxicity Test (Embryo-Larvae)

This guideline is unique to OPPTS and is based on OPP 72-3. The guideline has been improved with more elaborate test guidance provided. It is consistent with the ASTM Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs, E 724-89.

850.1075 Fish Acute Toxicity Test, Freshwater and Marine

This guideline has been modified from 40 CFR 797.1400 and OPP 72-1 and 72-3 and harmonized with OECD guideline 203. The key modification establishes a minimum of 7 fish per test level.

850.1085 Fish Acute Toxicity Mitigated by Humic Acid

This guideline is unique to OPPTS and is substantively identical to 40 CFR 797.1460.

850.1300 Daphnid Chronic Toxicity Test

This guideline has been modified from 40 CFR 797.1330 and OPP 72-4 to be harmonized with OECD guideline 202. This guideline differs from the existing OECD 202 in that it establishes 21 days as the test duration (consistent with OPPT and OPP guidelines and draft OECD guidelines) and allows tests with 10 individually separated daphnids per test level (consistent with draft OECD guidelines).

850.1350 Mysid Chronic Toxicity Test

This guideline is based on 40 CFR 797.1950 and OPP 72-4 and is harmonized for use within OPPTS.

850.1400 Fish Early-life Stage Toxicity Test

This guideline based on OECD guideline 210 and is also consistent with 40 CFR 797.1600 and OPP 72-4.

850.1500 Fish Life Cycle Toxicity

This guideline is unique to OPPTS and is based on OPP 72-5.

850.1710 Oyster BCF

This guideline is based on 40 CFR 797.1830 and OPP 72-6 and is harmonized within OPPTS.

850.1730 Fish BCF

Awaiting final version of OECD guideline 305.

**850.1735 Whole Sediment Acute Toxicity Invertebrates,
Freshwater**

and

**850.1740 Whole Sediment Acute Toxicity Invertebrates,
Marine**

The Agency has developed two standardized test methods for determining sediment toxicity to

either freshwater or marine benthic organisms. Both test protocols, "USEPA Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants With Freshwater Invertebrates" (EPA 600\R-94\024) and "USEPA Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods" (EPA 600\R-94\025), have been harmonized within OPPTS and will be used to determine the toxicity of pesticides or toxics in sediment to the midge, *Chironomus tentans* or amphipod *Hyalella azteca* in freshwater or to either of the following four amphipods in estuarine/marine waters: *Ampelisca abdita*, *Eohaustorius estuarius*, *Rhepoxynius abronius*, and *Leptocheirus plumulosus*.

850.1790 Chironomid Sediment Toxicity Test [850.1790 is proposed new number.]

This guideline is based on 40 CFR 795.135 and 40 CFR 797.4050. It was formerly grouped with OPPT's vertebrate (tadpole) sediment guideline.

850.1800 Tadpole/Sediment Subchronic Toxicity Test [850.1800 is proposed new number for this guideline.]

This guideline is based on 40 CFR 797.4200 and 40 CFR 797.1995.

850.1850 Aquatic Food Chain Transfer

This guideline is unique to OPPTS and is based on OPP 72-6.

850.1900 Generic Aquatic Microcosm Test, Laboratory

This guideline is unique to OPPTS and is based on 40 CFR 797.3050.

850.1925 Site-Specific Aquatic Microcosm Test, Laboratory [850.1925 is proposed new number for this guideline.]

This guideline is unique to OPPTS and is based on 40 CFR 797.3100. It was formerly grouped with the Generic Aquatic Microcosm Test, Laboratory under 850.1900.

850.1950 Field Testing for Aquatic Organisms

This guideline is unique to OPPTS and is based on OPP 72-7 and the Agency's technical guidance document, EPA Publication No. EPA 540/09-88-035.

850.2100 Avian Acute Oral Toxicity Test

This guideline is unique to OPPTS and is based on 40 CFR 797.2175 and OPP 71-1.

850.2200 Avian Dietary Toxicity Test

This guideline has been modified from 40 CFR 797.2050 and OPP 71-2 to be harmonized with OECD guideline 205. The modification allows the use of Japanese quail as an acceptable test species, but retains a narrow range for the acceptable age of birds at test initiation which differs from OECD 205. A narrow range is necessary to ensure active feeding during the exposure phase of the test.

850.2300 Avian Reproduction Test

This guideline has been modified from 40 CFR 797.2130 and 797.2150 and OPP 71-4 to be harmonized with OECD guideline 206. However, the OECD guidelines identifies Japanese quail as an acceptable species, but retains a minimum of 10 weeks of exposure to the test material before onset of egg laying. This and other protocol details in the OECD guideline are not possible for Japanese quail. The report of an OECD workshop in Pensacola Florida has noted the need for comparative toxicity testing in Bobwhite, Mallard and Japanese quail. A new OECD guideline is being drafted for use with shorter half-life chemicals and Japanese quail. Validation and ring testing will most likely be needed before the guideline is finalized and accepted internationally. In light of these facts, the US is listing only Mallard and Bobwhite as acceptable species for avian reproduction testing at this time.

850.2400 Wild Mammal Acute Toxicity

This guideline is unique to OPPTS and is based on OPP 71-3.

850.2450 Terrestrial Microcosm Test

This guideline is unique to OPPTS and is based on 40 CFR 797.3775.

850.2500 Field Testing for Terrestrial Wildlife

This guideline is unique to OPPTS and is based on OPP 71-5 and the Agency's technical guidance document EPA 540/09-88-109.

850.3020 Honey Bee Acute Contact Toxicity Test

This guideline is unique to OPPTS and is based on OPP 141-1. This guideline has been substantively amended by the addition of procedures for conducting the test (based on standard methods in use in US laboratories.)

850.3030 Honey Bee-Toxicity of Residues on Foliage

This guideline is unique to OPPTS and is based on OPP 141-2.

850.3040 Field Testing for Pollinators

This guideline is unique to OPPTS and is based on OPP 141-5.

850.4000 Background - Nontarget Plant Testing

This guideline provides general guidance useful for performing plant testing and is based on guidance found in OPP 120-1, 120-2, and 120-3. Also expanded guidance and clarification have been provided in the areas of cultural practices for all tests.

850.4025 Target Area Phytotoxicity

This guideline is unique to OPPTS and is based on OPP 121-1. The guideline has been reworked and improved throughout. A requirement for use of better cultural practices to control insect, weed, or disease pests other than the target pest has

been added.

850.4100 Terrestrial Plant Toxicity Tier I,(Seedling Emergence)

This guideline is based on OPP 122-1. Greater flexibility is now allowed in species selection and specific guidance is provided for germination rates.

850.4150 Terrestrial Plant Toxicity, Tier I (Vegetative Vigor)

This guideline is unique to OPPTS and is based on OPP 122-1. Greater flexibility is now allowed in species selection.

850.4200 Seed Germination/Root Elongation Toxicity Test [Proposed new number for this guideline]

This guideline is based on 40 CFR 797.2750 and is unique to OPPT.

850.4225 Seedling Emergence, Tier II

This guideline is unique to OPPTS and is based on 40 CFR 797.2750 and 797.2800 and OPP 123-1. This guideline now provides more specific guidance with respect to germination criteria, use of seed treatments, formulation testing, routine watering of pots, and other cultural practices. It also allows more flexibility in selection of test species.

850.4230 Early Seedling Growth Toxicity Test [Proposed new number for this guideline.]

This guideline is unique to OPPT and is based on 40 CFR 797.2800. It differs from OPP guideline 122-1 in several critical areas, e.g. test article, number and range of dose levels, appropriate culture medium and is considered more relevant for testing industrial chemicals.

850.4250 Vegetative Vigor, Tier II

This guideline is unique to OPPTS and is based on 40 CFR 797.2750 and 797.2800 and OPP 123-1. This guideline now provides more specific guidance with respect to germination criteria, use of seed

treatments, formulation testing, routine watering of pots, and other cultural practices. It also allows more flexibility in selection of test species.

850.4300 Terrestrial Plants Field Study, Tier III

This guideline is based on OPP 124-1. New references providing general guidance for field practices have been added.

850.4400 Aquatic Plant Toxicity Test Using *Lemna* spp., Tiers I and II

This guideline is unique to OPPTS and is based on 40 CFR 797.1160 and OPP 122-2 and 123-2. The guideline has been modified and shortened in duration from a 14 day test to a 7 day test. The 7 day test guidance is fashioned after ASTM test guidance E 1415-91, "Guide for Conducting Static Toxicity Tests with *Lemna gibba*." The new guidance also allows testing with *Lemna gibba* or *Lemna minor*. Tiers I and II are now combined in one guidance document.

850.4450 Aquatic Plants Field Study, Tier III

This guideline is unique to OPPTS and is based on OPP 124-2. New references providing general guidance for field practices have been added.

850.4600 *Rhizobium* - Legume Toxicity

This guideline is unique to OPPTS and is based on 40 CFR 797.2900.

850.4800 Plant Uptake and Translocation Test

This guideline is unique to OPPTS and is based on 40 CFR 797.2850.

850.5100 Soil Microbial Community Toxicity Test

This guideline is unique to OPPTS and is based on 40 CFR 797.3700.

850.5400 Algal Toxicity Test, Tiers I and II

This guideline has been harmonized within OPPTS

and is based on 40 CFR 797.1050 and OPP 123-2. This guideline differs from OECD guideline 201 in that it prohibits aeration, broadens the list of acceptable species, and requires a 96 hour exposure duration. In addition, the revised guideline has been reviewed in an independent testing laboratory to refine the test protocol throughout.

Although there is an OECD test guideline for algal toxicity testing, it calls for a test duration of only 72 hours which is only suitable if light intensity, inoculum levels and temperature are all stepped up beyond natural conditions. Under these special conditions, the algae will achieve fast growth, but the resulting test becomes a highly artificial screen. OECD intends to reexamine its test protocol for algal toxicity testing. In contrast, the OPPTS harmonized test calls for dose response testing under a broad range of doses, including pesticide label rates, under natural conditions but with the longer test duration. Guidance also is provided for a dose response test for risk assessment purposes.

850.6200 Earthworm Subchronic Toxicity Test

This guideline is unique to OPPT and is based on 40 CFR 795.150. It establishes the test duration as 28 days, uses only artificial soil as the test medium, and specifies the test species as *Eisenia fetida andrei* (Bouche).

850.6800 Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble Chemicals

This guideline is based on 40 CFR 795.170 and OECD guideline 209.